ESTER-MEDIATED NITROSAMINE FORMATION FROM NITRITE AND SECONDARY OR TERTIARY AMINES

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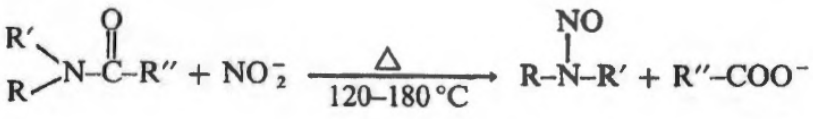
SUMMARY

N-Nitrosamines are formed from the heating of either a secondary or a tertiary amine with sodium nitrite in the presence of a high-boiling ester such as 2-acetoxyethanol in ethylene glycol. The four secondary and six tertiary amines examined were found to produce *N*-nitrosamines in yields ranging from 4% to 80% when equimolar amounts of amine and ester were heated at 120 °C with one- to ten-fold equivalents of sodium nitrite in ethylene glycol. Secondary amines competitively produced acetamides at a rate slightly greater than *N*-nitrosamine formation. Preincubation of a large excess of sodium nitrite and ester led to the rapid formation of *N*-nitrosamines in high yield. The reaction of tribenzylamine resulted in the formation of both benzaldehyde and dibenzylnitrosamine. *N,N*-Dimethylbenzylamine reacted to give nearly equimolar amounts of *N*-nitrosodimethylamine and *N*-nitroso-*N*-methylbenzylamine. It is proposed that the nitrosating agent is a nitrous ester, and it is shown that 2-benzoxyethyl nitrite rapidly nitrosates secondary and tertiary amines under these reaction conditions. It is also proposed that these transformations are good models for the environmental formation of *N*-nitrosamines in foods and commercial products.

INTRODUCTION

We reported previously that *N,N*-dialkylamides react with sodium nitrite upon heating to give variable yields of *N*-nitrosamines (0.3–27%), as indicated in equation (1) (Loeppky *et al.*, 1982).



(1)

We found that both the yield and rate of *N*-nitrosamine formation were markedly increased upon the introduction of ethylene glycol or glycerol. Additional experimentation in our laboratory has permitted us to postulate a mechanism for this reaction and the unusual role

of the polyhydric solvents. The first step involves the transfer of an acyl group from the amide to the alcohol function of the solvent. The resulting ester is attacked by ionic nitrite to yield the nitrosating species, an alkyl nitrite. The nitrous ester then reacts with the amine produced in the first transformation, to give the *N*-nitrosamine. In the course of verifying this hypothesis we have investigated the reaction of secondary amines with the acetate esters of ethylene glycol and sodium nitrite in ethylene glycol. These experiments have been performed to answer the question: can esters and ionic nitrite lead to extensive nitrosation of secondary and tertiary amines? The results presented in this paper demonstrate that the answer is yes. Moreover, we believe that this general reaction scheme is mainly responsible for the production of *N*-nitrosamines in cosmetics, metal-working fluids, shampoos and other toiletry articles, as well as certain cooked and cured meats.

EXPERIMENTAL

Secondary amine nitrosation

The nitrosation reactions were conducted either in flasks connected to a reflux condenser, which was sealed at the top with a rubber septum, or in a septum-sealed vial. The flasks were heated in an oil bath at 120 °C and the vials were heated in a metal block at the same temperature. All experiments employed ethylene glycol as a solvent and sodium nitrite, ethylene glycol diacetate and the desired amine as reactants. Reactions were conducted in an atmosphere of argon. A typical procedure involved the heating of 0.306 g (4.43 mmol) sodium nitrite, 0.29 g (4.4 mmol) ethylene glycol diacetate and 30 mL ethylene glycol in a 50-mL flask for 2.7 h at 120 °C. Pyrrolidine (0.33 g, 4 mmol) was added by syringe below the surface of the hot liquid mixture. Samples were taken through the septum at specific intervals. When the reactions were conducted in vials, each point in the kinetic analysis was obtained from a separate vial, the contents of which were 'quenched' as described below.

Tertiary amine nitrosation

Tertiary amine nitrosations were conducted in a manner similar to the secondary amine nitrosation, except that the preincubation of the solvent, ester and sodium nitrite, was found to have little effect on the time-course of the transformation and was therefore eliminated. A typical procedure follows: two solutions were prepared. Solution 1 consisted of 0.9523 g (6.52 mmol) ethylene glycol diacetate and 0.4405 g (6.38 mmol) sodium nitrite, dissolved in enough ethylene glycol to bring the volume to 25.0 mL. Solution 2 consisted of a 5.37×10^{-2} mol/L solution of tribenzylamine in ethylene glycol (a saturated solution). Solutions 1 and 2 were brought to 120 °C in an oil bath. Reaction vials were charged with 1.50 mL of solution 1 and 0.50 mL of solution 2, sealed with Teflon-lined caps and heated at 120 °C, with occasional shaking. Sample vials were removed from the heating block at specific times, cooled rapidly, and 50-μL samples were taken and added to methanol in a 10-mL volumetric flask. After the samples had been diluted to a volume of 10.0 mL, analysis was performed by high-pressure liquid chromatography (HPLC), as indicated below.

Nitrosation of tribenzylamine with 2-benzoxyethyl nitrite

Tribenzylamine (0.013 g, 0.045 mmol) was weighed into each of 12 vials. The co-reactant was prepared by diluting 2.028 g of 55% 2-benzoxyethyl nitrite with 50.0 mL ethylene glycol in volumetric flask. Four mL of the solution was then placed in each vial. The vial headspace was purged with argon, and the vials were sealed. They were then heated at 120 °C, removed at

specific times, cooled and diluted to 25.0 mL with methanol. Analyses were performed by HPLC, as described below.

Analyses

The majority of *N*-nitrosamine analyses were performed by reversed-phase HPLC, using a Waters instrument, equipped for gradient elution and employing an autosampler with a variable-volume injection system and an ultra-violet detector, operating at 254 nm. As an example, *N*-nitrosopyrrolidine (NPYR) was determined in the quenched sample, diluted to standard volume with methanol, by injection (10 µL) onto a duPont Zorbax-CN column (4.6 mm × 25 cm) which was eluted with methanol: water (2 mL/min at 20:40 to 40:60–4 min). The elution volume for NPYR was 6.4 mL and quantification was carried out using a standard curve obtained with external standards. Each point represents the repetition of three determinations. Benzaldehyde produced in the nitrosation of tribenzyl amine was also determined by HPLC, using a similar technique. Specific details of the chromatographic determination of various compounds can be obtained from the authors.

N-Acetylpyrrolidine and NPYR were analysed by gas chromatography (GC) as well. Samples from the nitrosation of pyrrolidine were prepared by passing a 0.5-mL sample through a small column containing 2 mL of AG 1-x8 anion-exchange resin with distilled water until 10.0 mL had been collected. The amide and nitrosamine were detected by a flame-ionization detector (FID) using a 2-m OV-225 column for separation (75 °C/min; 5 °C/min to 100 °C; 10 °C/min to 250 °C). Under these conditions, the retention time for NPYR was 6.24 min and that of *N*-acetylpyrrolidine 7.5 min. Nitrite ion consumption in this experiment was determined spectrophometrically, using Shin's reagent.

RESULTS

Secondary amine nitrosation

Typical nitrosation experiments were performed by heating the desired amine and sodium nitrite with an equilibrium mixture of monoacetyl and diacetyl ethylene glycol in ethylene glycol in a sealed container. The ester was added as diacetyl ethylene glycol and pre-equilibrated with ethylene glycol to give the mixture of esters, which was composed principally of monoacetyl ethylene glycol. Initial experiments were conducted at 180 °C but a reaction temperature of 120 °C was found to be much better for convenient study of the transformations, and all subsequent reactions were done at this temperature. Figure 1 demonstrates that the ester-mediated nitrosation of pyrrolidine is much faster and more extensive than the production of this nitrosamine from *N*-acetylpyrrolidine or the parent amine under the same conditions. The amine in these reactions is involved in two competitive transformations – nitrosation and reversible *N*-acylation. The equilibrium significantly favours amide formation (*N*-acylation) under the reaction conditions. The product ratio of *N*-nitrosamine to amide can be manipulated by adjusting the initial reaction conditions. The time course of a typical transformation involving pyrrolidine is shown in Figure 2. In this case, the ester and the sodium nitrite were preincubated at 120 °C for 2.5 h, prior to introduction of the amine. The results of a similar experiment employing a 25-fold excess of ester and sodium nitrite are shown in Figure 3. It can be seen that it led to very rapid and extensive formation of *N*-nitrosopyrrolidine.

A number of amines of varying basicity and structure have been nitrosated by the general procedure described above. The results are reported in Table 1. Generally, we made no attempt to maximize the yield of *N*-nitrosamine, since we have demonstrated that this can be done by manipulating the initial reactant concentrations and the reaction conditions. The

Fig. 1. Formation of *N*-nitrosopyrrolidine (NPYR) from the heating of 1.5 mol/L amine or amide and sodium nitrite at 180°C in glycol; ♦, amine + ROAc + NO_2^-; ●, amide + NO_2^-; ▲, amine + NO_2^-.

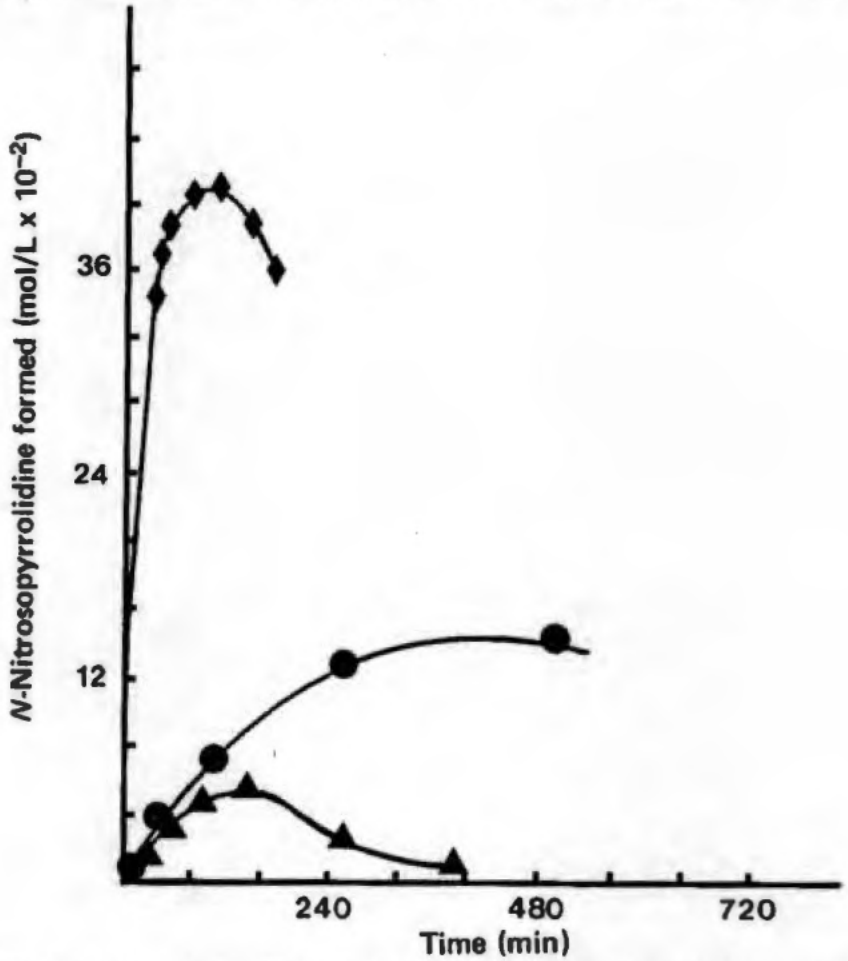


Fig. 2. % Formation of *N*-nitrosopyrrolidine (■) and *N*-acetylpyrrolidine (○) and % consumption of NO_2^- (●) as a function of time (Table 1, entry 2).

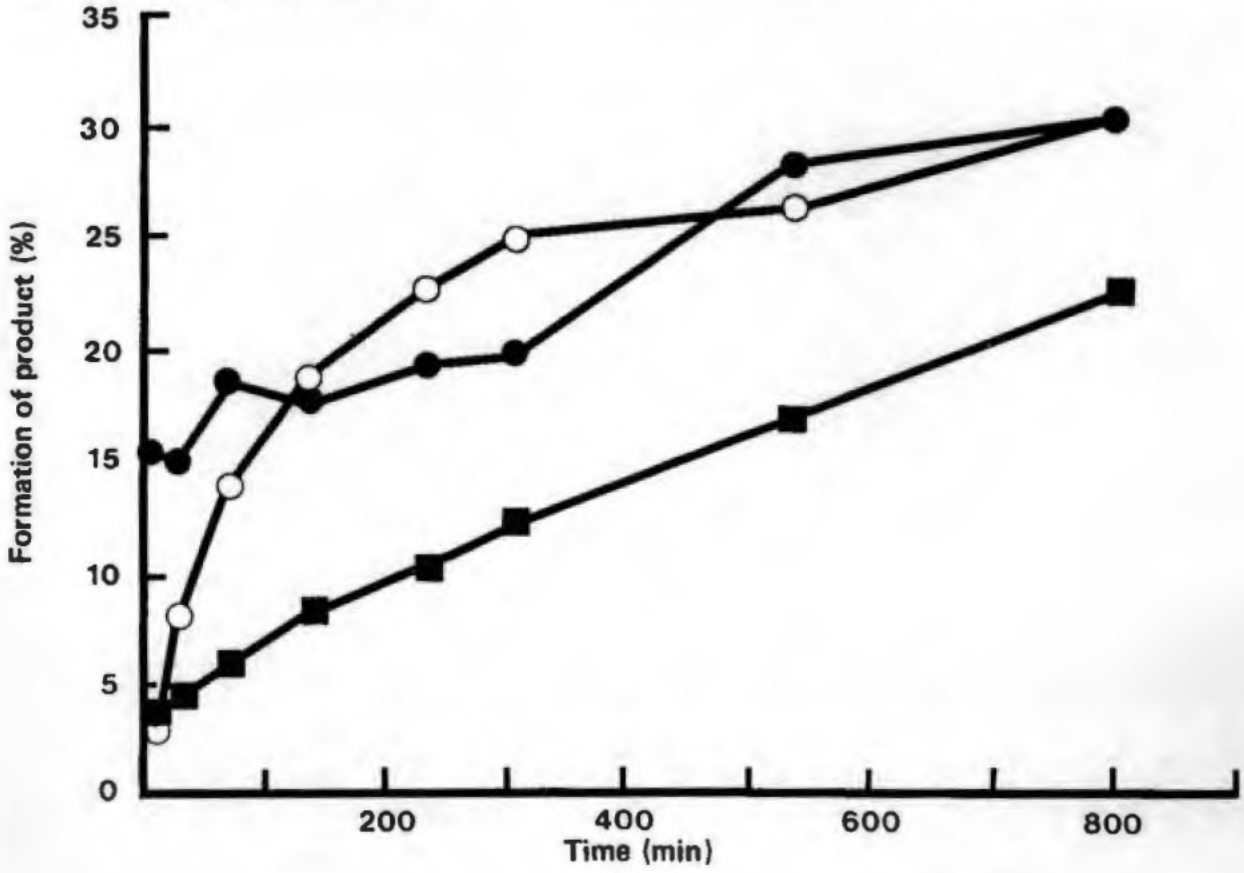


Fig. 3. % Formation of *N*-nitrosopyrrolidine as a function of time (Table 1, entry 1).

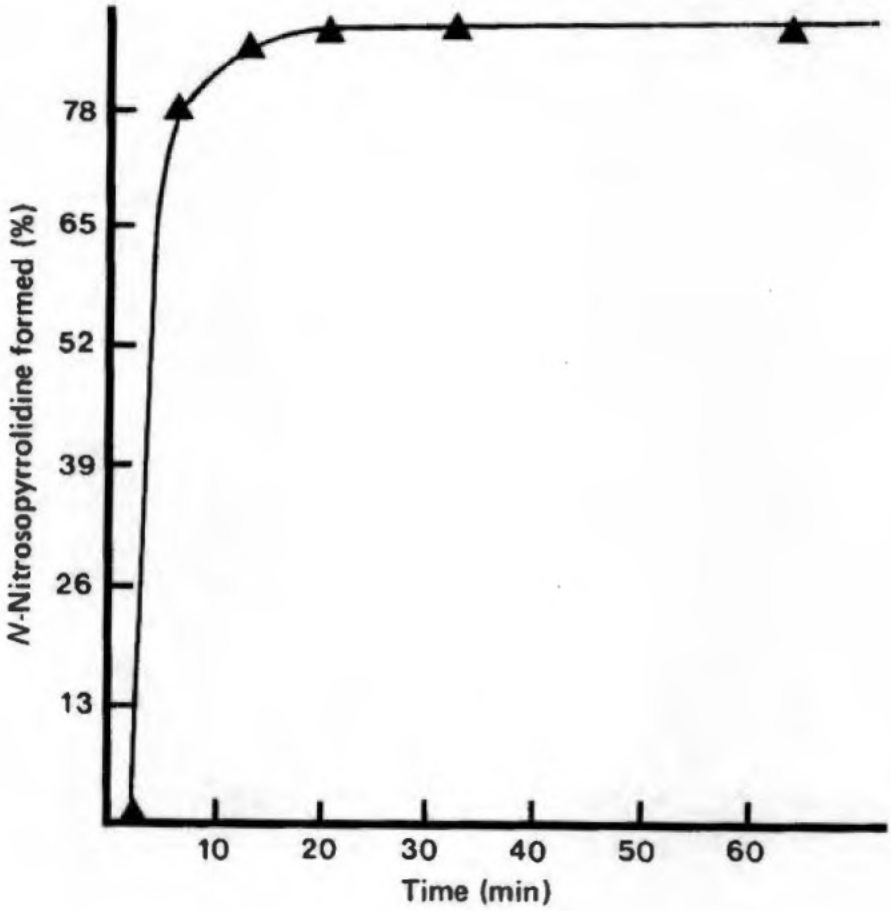


Table 1. Ester-mediated nitrosation of secondary amines

Entry	Amine	Reactant ratio[a]			Yield[b] (%/h)	$10^2 k'$[c] (min^{-1})
		Amine	Ester	Nitrite		
1	Pyrrolidine	1	25	25	67/12	139
2	Pyrrolidine	1	2.21	1.1	32/13.5	2.72
3	Diethyl	1	1.2	1	53/45	2.71
4	Diethanol	1	1.2	1	25/14.5	2.7
5	Dibenzyl	1	2.8	10	80/122	2.3

[a] [reactant]/[amine]
[b] % Yields based on amine at the time indicated
[c] Observed rate constants from plots of ln(% reaction) versus time

ester-mediated transformations of the amine to amide and *N*-nitrosamine are kinetically complex because of the parallel and competitive steps involving both the ester and the amine. A better understanding of the reaction mechanism will require further kinetic experiments, but manipulation of the data given in Figure 2 reveals the interesting feature that a plot of time *versus* (log % reaction) is linear for this transformation and for the other transformations noted in Table 1. In several instances, points at longer times had to be dropped, but the reaction obeys this kinetic law up to the percent reaction given in Table 1. The slopes of these plots are reported

as pseudo first-order rate constants in Table 1. An interesting feature of these rate constants is that, within experimental error, they are all equal (Table 1, entries 2–5), despite the differences in amine basicity and structure.

The nitrous ester, 2-acetoxyethyl nitrite, is a possible intermediate in this transformation and may be the active nitrosating agent (Loeppky *et al.*, 1982). Since the report by Challis and Shuker (1979), that secondary amines can be nitrosated by activated nitrous esters, we have synthesized 2-acetoxyethyl nitrite and 2-benzoxyethyl nitrite and shown that both compounds nitrosate secondary amines very rapidly at 120 °C. The latter ester is purified more easily than the former.

Tertiary amine nitrosation

The heating of tribenzylamine, sodium nitrite and diacetyl glycol in ethylene glycol at 120 °C in a sealed vial results in the slow production of equal molar quantities of *N*-nitrosodibenzylamine and benzaldehyde (equation 2).

$$(\varnothing CH_2)_3N + NaNO_2 + AcOCH_2CH_2OAc \xrightarrow[OH\quad OH]{\overset{\triangle}{120}} \varnothing\overset{O}{\overset{\|}{C}}-H + (\varnothing CH_2)_2N-NO \qquad (2)$$

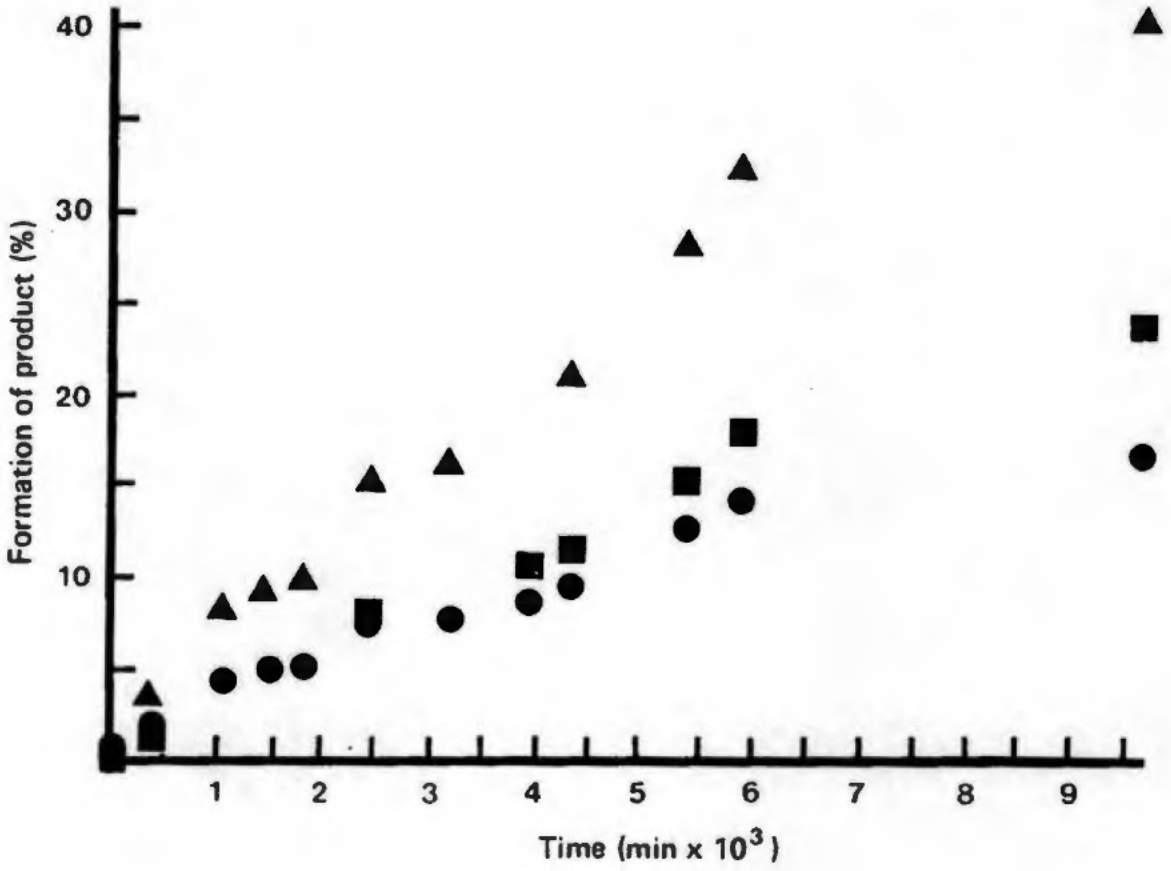
The reaction must be protected from light in order to avoid the photodecomposition of *N*-nitrosodibenzylamine, which also gives benzaldehyde. Five other structurally-varied tertiary amines have been shown to produce *N*-nitrosamines when heated with sodium nitrite under these conditions. The data are given in Table 2. The time *versus* % reaction data were treated in a manner similar to those for the secondary amines, and the apparent rate constants, although considerably less precise, are reported. It can be seen that the apparent rate varies with the structure of the starting amine. *N,N*-Dimethylaniline was quite reactive, while *N*-butylpyrrolidine was relatively unreactive under the conditions of the transformation. Tribenzylamine represented the only case in which we attempted to identify the product cleaved from the amine nitrogen.

Table 2. Ester-mediated nitrosation of tertiary amines

Entry	Amine	Reactant ratio[a]			Yield[b] (%/h)	10^5 k[c] (min^{-1})
		Amine	Ester	Nitrite		
1	Tributyl	1	1.28	4.88	22/14	3.0
2	*N,N*-Dimethylaniline	1	1.1	4.59	45/14	7.0
3	*N*-Butylpyrrolidine	1	1.19	4.85	4.1/91	–
4	*N,N*-Dimethylbenzyl (NMBzA)	1	1.22	5.0	24/161	2.9
5	*N,N*-Dimethylbenzyl (NDMA)	1	1.22	5.0	17/161	1.8
6	Triethyl	1	1.2	1.0	17/26	1.0
7	Tribenzyl	1	2.83	10.2	7.5/33	4.1

[a] [reactant]/[amine]
[b] % Yields based on amine in the time indicated
[c] Approximate observed rate constants determined from plots of ln (% reaction) versus time. These constants should be used only as a rough gauge of reactivity, since it is not clear that the reaction exhibits pseudo first-order kinetics.

Fig. 4. % Formation of *N*-nitrosodimethylamine (●) and *N*-nitroso-*N*-methylbenzylamine (■) from ester-mediated nitrosation of *N,N*-dimethylbenzylamine as a function of time. ▲, sum of % yields.



N,N-Dimethylbenzylamine reacted with sodium nitrite in the presence of the ester to produce both *N*-nitrosodimethylamine (NDMA) and *N*-nitroso-*N*-methylbenzylamine (equation 3).

$$\varnothing CH_2N(CH_3)_2 + NaNO_2 + AcOCH_2CH_2OAc \xrightarrow[120]{\triangle} (CH_3)_2NNO + \varnothing CH_2\overset{\overset{NO}{|}}{N}CH_3 \qquad (3)$$

OH OH

The time course of this transformation is shown in Figure 4. It can be seen that NDMA and *N*-nitroso-*N*-methylbenzylamine are formed in approximately equal molar amounts throughout the course of the reaction.

Since secondary amines are proposed intermediates in the acidic nitrosation of tertiary amines, we examined the reaction of tribenzylamine for the presence of dibenzylamine or its *N*-acetyl derivative. Neither of these substances was found. As was discussed in the case of secondary amine nitrosation, we have hypothesized that a nitrous ester might be the effective nitrosating agent in these transformations. Because of the difficulties we encountered in the preparation of 2-acetoxyethyl nitrite, we worked here with 2-benzyloxyethyl nitrite, which was

much more easily prepared and purified. This nitrous ester reacted rapidly with tribenzylamine to form *N*-nitrosodibenzylamine, but no significant quantity of benzaldehyde (equation 4).

$$\text{Ø–}\overset{\overset{\text{O}}{\|}}{\text{C}}\text{–OCH}_2\text{CH}_2\text{–O–N=O} + (\text{ØCH}_2)_3\text{N} \xrightarrow[120]{\Delta} (\text{ØCH}_2)_2\text{NNO} + ? \qquad (4)$$

When a 10-fold molar excess of the ester was employed, the reaction exhibited pseudo first-order rate behaviour up to 89% production of *N*-nitrosodibenzylamine (reaction time 2.3 h). The observed rate constant under these conditions was $(1.3 \pm 0.2) \times 10^{-2}$ min^{-1}.

DISCUSSION

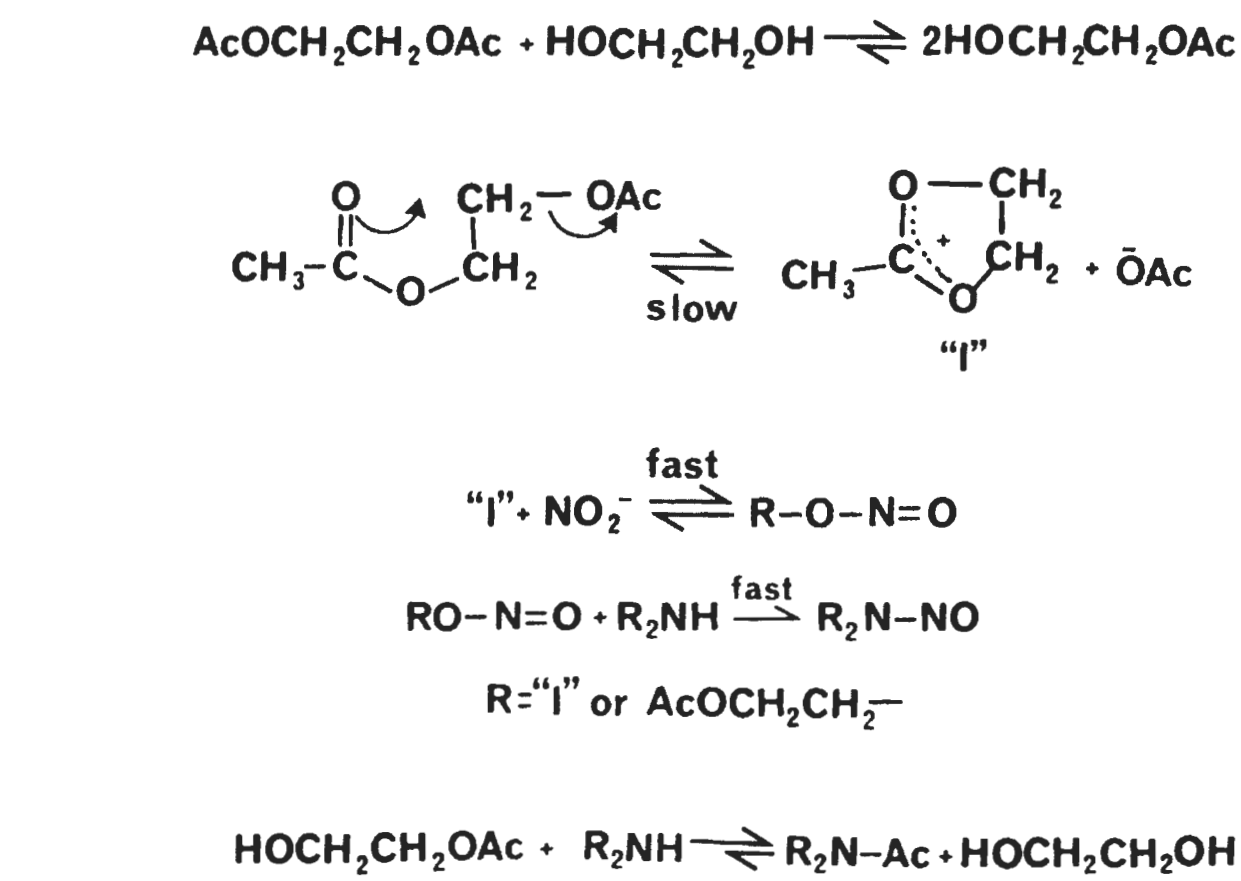
The results described above provide ample evidence that acetate esters of ethylene glycol promote the nitrosation of both secondary and tertiary amines by ionic nitrite. *N*-Nitrosamine formation has been shown to result from a wide range of structurally varied amines of different basicity. The nitrosation of secondary amines occurs with competitive formation of amides from the ester, but this reaction is reversible, and we have demonstrated that heating of amides with ionic nitrite in ethylene glycol or glycerine results in *N*-nitrosamine formation.

While the reaction has not been investigated from the perspective of the structure of the ester, our preliminary investigations suggest that esters of high-boiling alcohols will be effective in promoting the nitrosation of amines by the scheme discussed here. Methyl and ethyl esters of carboxylic acids are not anticipated to be effective in promoting nitrosation reactions, because the alkyl nitrites formed from them are so volatile. We observed this previously when examining the reaction between sodium nitrite and ethyl-*N*-acetylproline (Loeppky *et al.*, 1982).

The data on secondary amine nitrosation reactions presented here strongly suggest that production of the nitrosating agent is the rate-limiting step in this transformation. The data of Table 1 support this hypothesis. Structurally different amines gave similar rate constants for nitrosation. Preincubation of nitrite with the acetate ester led to more rapid nitrosation of the amine. Preincubation of relatively large amounts of ester and nitrite (compared to amine) prior to introduction of the amine led to rapid and extensive *N*-nitrosamine formation. The precise nature of the nitrosating agent or agents under these conditions is not yet known. We have shown that a nitrous ester of ethylene glycol is capable of nitrosating both secondary and tertiary amines under the reaction conditions described and that the reaction takes place rapidly. It is also possible, however, that the reaction involves a species such as that shown in Figure 5.

The data on tertiary amine nitrosation display several interesting features. Tribenzylamine nitrosation follows the same course as it does in acidic media, producing both *N*-nitrosodibenzylamine and benzaldehyde (Smith & Leoppky, 1967). The reaction, however, must occur by a different mechanism than that involved in the acidic nitrosation of tertiary amines. Several lines of evidence point to this conclusion. First of all, the nitrosation of *N*-nitroso-*N*-methylbenzylamine and NDMA. We have demonstrated that the acidic nitrosation of this same amine results in the preferential formation of the former *N*-nitrosamine at a three-to-one ratio. This altered selectivity in the cleavage of the alkyl group from nitrogen is strongly suggestive of a new pathway for the nitrosation reaction. The acidic nitrosation of tribenzylamine and other tertiary amines investigated by Smith and Loeppky (1967) involves an intermediate *N,N*-dialkylimminium ion. This imminium ion is hydrolysed by the water in the acidic solution to produce a secondary amine and an aldehyde. The secondary amine is then

Fig. 5. Scheme for nitrosating reactions of a nitrous ester with secondary and tertiary amines.



presumed to be nitrosated to give the *N*-nitrosamine. We have not been able to detect secondary amines or their amides as intermediates in the nitrosation of tertiary amines by esters and ionic nitrite. Lijinsky *et al.* (1972) and Keefer and Roller (1973) have suggested an alternative mode of *N*-nitrosamine formation from an imminium ion, which could account for the formation of both *N*-nitrosodibenzylamine and benzaldehyde in this reaction. The imminium ion produced by the elimination of NOH from the nitrosated tertiary amine is envisioned to react with ionic nitrite to generate an α-aminonitrous ester. This species is presumed to decompose directly to the *N*-nitrosamine and the aldehyde. It is interesting that the nitrosation of tribenzylamine with 2-benzoxyethyl nitrite does not result in the production of benzaldehyde, although it does give *N*-nitrosodibenzylamine. This suggests yet another mode of tertiary amine nitrosation. Obviously, more work must be done before a detailed understanding of this reaction mechanism and the nature of the nitrosating agent become evident. In spite of this, the elaboration of these reactions provides a missing link in the environmental production of *N*-nitrosamines from ionic nitrite.

There have been numerous reports of environmental *N*-nitrosamine formation in which the reactants have not encountered an acidic environment. Previous work by Keefer and Roller (1973), Croisy *et al.* (1980), Challis and Shuker (1979), Challis and Outram (1979) and Challis and Kryptopoulos (1979) have demonstrated how *N*-nitrosamine formation can occur in an alkaline medium. Keefer's group has demonstrated how formaldehyde and metal ions can catalyse nitrosation, while Challis' group has demonstrated how oxides of nitrogen and nitrous esters can be effective nitrosating agents in alkaline media. Although it is difficult to be sure that *N*-nitrosamine formation is occuring in the absence of any oxides of nitrogen, there are many cases of environmental *N*-nitrosamine occurrence and formation which involve neither acidic nor alkaline aqueous mixtures.

Fig. 6. Scheme for *N*-nitrosamine formation in the cooking of nitrite-cured meats.

GLYCERIDE + H_2O → GLYCEROL + ACID

GLYCEROL + PEPTIDE → AMINE + ESTER

GLYCERIDE + NITRITE → GLYCERYL NITRITE + CARBOXYLATE

GLYCERYL NITRITE + AMINE → NITROSAMINE + GLYCEROL

Probably the best-known example involves the formation of NPYR in the cooking of nitrite-cured meats. These meats, of course, contain esters of glycerol (fat). We believe that *N*-nitrosamine formation occurs by the route we have discussed in this paper and which is sketched out in Figure 6. Cosmetic and metal-working fluid formulation often contain *N*-nitrosamines formed from alkanolamines and frequently contain either amides, esters or fatty acids. Temperatures as high as 100 °C are often reached during the formulation of these materials. One can understand how trace amounts of *N*-nitrosamines could be formed from adventitious nitrite or nitrite formed from the hydrolysis of oxides of nitrogen under the alkaline conditions present in metal-working fluids, for example. It is also possible that this general route could apply to the formation of *N*-nitrosamines during tobacco smoking. If the active nitrosating agent in these reactions is a nitrous ester, there is good reason to believe that this type of nitrosation reaction can be inhibited relatively easily. Nitrous esters are very reactive substances and may be chemically scavanged more easily than nitrous acid, and certainly more easily than ionic nitrite. Our research is proceeding on this subject.

RECOMMENDATION

We recommend that the IARC sponsor the development of a nitrosation test similar to the WHO acidic nitrosation test. This test would involve heating formulations at 100 °C for a fixed time, followed by analysis for *N*-nitrosamines.

ACKNOWLEDGEMENT

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